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PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re: Caroline CONNELLY et al.

Int'l Appl. No.: National Stage of PCT/GB01/01615 Group Art Unit: [Not Assigned]

Filed: June 4, 2001 Examiner: [Not Assigned]

For: HOMOCYSTEINE ASSAY

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, DC 20231

Sir:

Prior to calculating the filing fee for the above-identified patent application,
please amend the application as follows:

In the Claims:

Please cancel claims 16 and 17 without prejudice, the subject matter of which has been
incorporated into claim 15.

Please amend claims 4, 7-11, 13-15, 19, and 20 as follows:

4. (Amended) A homocysteine assay which comprises contacting a biological
fluid sample with a homocysteine converting enzyme, characterised in that before contact
with said enzyme said sample is treated with an agent which serves to deactivate pyruvates [,
e.g. by immobilizing, binding or converting pyruvates].

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7. (Amended) An assay [as claimed in] which comprises at least two [of claims 1 to 6] assays selected from the group consisting of:

a homocysteine assay which comprises contacting a biological fluid sample with a liquid reagent containing a homocysteine converting enzyme, wherein said reagent is produced by adding an aqueous liquid to a lyophilisate containing said enzyme and a cryo/lyoprotectant, characterised in that said lyophilisate is substantially free of thiol-containing cryo/lyoprotectants;

a homocysteine assay which comprises contacting a biological fluid sample with a liquid reagent containing homocysteine desulphurase, wherein said liquid reagent is an aqueous liquid containing homocysteine desulphurase, a thiol-reducing reagent, and a proteinaceous or non-proteinaceous stabilizer;

a homocysteine assay which comprises contacting a biological fluid sample with a homocysteine converting enzyme, characterised in that before contact with said enzyme said sample is treated with an agent which serves to deactivate pyruvates;

a homocysteine assay which comprises contacting a biological fluid sample with an immobilized homocysteine converting enzyme, wherein said biological fluid sample contacts the immobilized enzyme under such time and conditions to allow the homocysteine in the sample to bind to said enzyme, characterised in that the biological fluid sample is then removed from the assay; and

a homocysteine assay which comprises contacting a biological fluid sample with a homocysteine converting enzyme, characterized in that before contact with said enzyme said sample is filtered through an exclusion filter and centrifuged in order to remove pyruvates.

8. (Amended) An assay [as claimed in] which comprises at least three [of claims 1 to 6] assays selected from the group consisting of:

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a homocysteine assay which comprises contacting a biological fluid sample with a liquid reagent containing a homocysteine converting enzyme, wherein said reagent is produced by adding an aqueous liquid to a lyophilisate containing said enzyme and a cryo/lyoprotectant, characterised in that said lyophilisate is substantially free of thiol-containing cryo/lyoprotectants;

a homocysteine assay which comprises contacting a biological fluid sample with a liquid reagent containing homocysteine desulphurase, wherein said liquid reagent is an aqueous liquid containing homocysteine desulphurase, a thiol-reducing reagent, and a proteinaceous or non-proteinaceous stabilizer;

a homocysteine assay which comprises contacting a biological fluid sample with a homocysteine converting enzyme, characterised in that before contact with said enzyme said sample is treated with an agent which serves to deactivate pyruvates;

a homocysteine assay which comprises contacting a biological fluid sample with an immobilized homocysteine converting enzyme, wherein said biological fluid sample contacts the immobilized enzyme under such time and conditions to allow the homocysteine in the sample to bind to said enzyme, characterised in that the biological fluid sample is then removed from the assay; and

a homocysteine assay which comprises contacting a biological fluid sample with a homocysteine converting enzyme, characterized in that before contact with said enzyme said sample is filtered through an exclusion filter and centrifuged in order to remove pyruvates.

9. (Amended) An assay [as claimed in] which comprises at least four [of claims 1 to 6] assays selected from the group consisting of:

a homocysteine assay which comprises contacting a biological fluid sample with a liquid reagent containing a homocysteine converting enzyme, wherein said reagent is

an assay for homocysteine which comprises contacting a biological fluid sample with a reducing agent and subsequently with homocysteine desulphurase, characterised in that said sample is contacted with an agent which binds, oxidizes or depotentiates said reducing agent after being contacted with said homocysteine desulphurase;

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a homocysteine assay which comprises contacting a biological fluid sample with a liquid reagent containing a homocysteine converting enzyme, wherein said reagent is produced by adding an aqueous liquid to a lyophilisate containing said enzyme and a cryo/lyoprotectant, characterised in that said lyophilisate is substantially free of thiol-containing cryo/lyoprotectants; and

a homocysteine assay which comprises contacting a biological fluid sample with a homocysteine converting enzyme, characterised in that before contact with said enzyme said sample is treated with an agent which serves to deactivate pyruvates.

11. (Amended) An assay as claimed in [claims 1, 3, and 4] claim 8 wherein said assay comprises

an assay for homocysteine which comprises contacting a biological fluid sample with a reducing agent and subsequently with homocysteine desulphurase, characterised in that said sample is contacted with an agent which binds, oxidizes or depotentiates said reducing agent after being contacted with said homocysteine desulphurase;

a homocysteine assay which comprises contacting a biological fluid sample with a liquid reagent containing homocysteine desulphurase, wherein said liquid reagent is an aqueous liquid containing homocysteine desulphurase, a thiol-reducing reagent, and a proteinaceous or non-proteinaceous stabilizer; and

a homocysteine assay which comprises contacting a biological fluid sample with a homocysteine converting enzyme, characterised in that before contact with said enzyme said sample is treated with an agent which serves to deactivate pyruvates.

a reducing agent [, e.g. dithiothreitol, dithioerythiol, TCEP or methyl iodide];

optionally a filter capable of removing red blood cells from blood.

Please add new claims 21-23 as follows:

23. (New) An assay as claimed in claim 6 wherein said homocysteine converting enzyme is HDS and wherein a NAD⁺/NADH cycling reaction is used to generate a coloured

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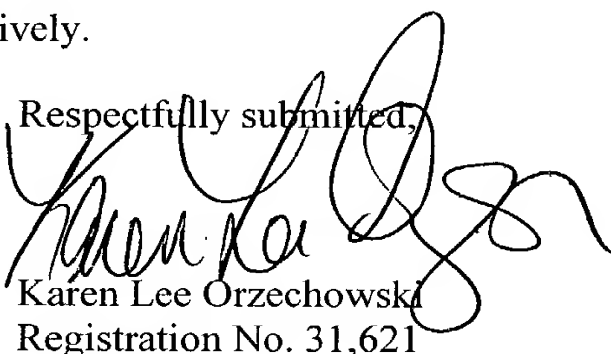
compound the concentration of which may be correlated to the concentration of homocysteine in the initial biological fluid sample.

REMARKS

The foregoing amendments have been made solely to eliminate multiple dependent claims and to place the claims in U.S. style claim format rather than the European style used in the International Application. No new matter has been added.

A clean copy of all of the pending claims and a copy of the marked up claims are attached hereto as Appendices A and B, respectively.

Respectfully submitted,


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I hereby certify that this correspondence is being deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to Assistant Commissioner For Patents, Washington, DC 20231, on June 4, 2001

Karen Lee Orzechowski

APPENDIX A
CLEAN COPY OF CLAIMS

2. A homocysteine assay which comprises contacting a biological fluid sample with a liquid reagent containing a homocysteine converting enzyme, wherein said reagent is produced by adding an aqueous liquid to a lyophilisate containing said enzyme and a cryo/lyoprotectant, characterised in that said lyophilisate is substantially free of thiol-containing cryo/lyoprotectants.

3. A homocysteine assay which comprises contacting a biological fluid sample with a liquid reagent containing homocysteine desulphurase, wherein said liquid reagent is an aqueous liquid containing homocysteine desulphurase, a thiol-reducing reagent, and a proteinaceous or non-proteinaceous stabilizer.

4. A homocysteine assay which comprises contacting a biological fluid sample with a homocysteine converting enzyme, characterised in that before contact with said enzyme said sample is treated with an agent which serves to deactivate pyruvates.

5. A homocysteine assay which comprises contacting a biological fluid sample with an immobilized homocysteine converting enzyme, wherein said biological fluid sample contacts the immobilized enzyme under such time and conditions to allow the homocysteine in the sample to bind to said enzyme, characterised in that the biological fluid sample is then removed from the assay.

6. A homocysteine assay which comprises contacting a biological fluid sample with a homocysteine converting enzyme, characterized in that before contact with said enzyme said sample is filtered through an exclusion filter and centrifuged in order to remove pyruvates.

7. An assay which comprises at least two assays selected from the group

an assay for homocysteine which comprises contacting a biological fluid sample with a reducing agent and subsequently with homocysteine desulphurase, characterised in that said sample is contacted with an agent which binds, oxidizes or depotentiates said reducing agent after being contacted with said homocysteine desulphurase;

a homocysteine assay which comprises contacting a biological fluid sample with a reagent containing a homocysteine converting enzyme, wherein said reagent is formed by adding an aqueous liquid to a lyophilisate containing said enzyme and a lyoprotectant, characterised in that said lyophilisate is substantially free of thiophilic cryo/lyoprotectants;

a homocysteine assay which comprises contacting a biological fluid sample with a reagent containing homocysteine desulphurase, wherein said liquid reagent is an aqueous liquid containing homocysteine desulphurase, a thiol-reducing reagent, and a proteinaceous or non-proteinaceous stabilizer;

a homocysteine assay which comprises contacting a biological fluid sample with a cysteine converting enzyme, characterised in that before contact with said enzyme sample is treated with an agent which serves to deactivate pyruvates;

a homocysteine assay which comprises contacting a biological fluid sample with an immobilized homocysteine converting enzyme, wherein said biological fluid sample is incubated with the immobilized enzyme under such time and conditions to allow the homocysteine in the sample to bind to said enzyme, characterised in that the biological fluid sample is then removed from the assay; and

a homocysteine assay which comprises contacting a biological fluid sample with a cysteine converting enzyme, characterized in that before contact with said enzyme sample is filtered through an exclusion filter and centrifuged in order to remove vesicles.

8. An assay which comprises at least three assays selected from the group

an assay for homocysteine which comprises contacting a biological fluid sample with a reducing agent and subsequently with homocysteine desulphurase, characterised in that said sample is contacted with an agent which binds, oxidizes or depotentiates said reducing agent after being contacted with said homocysteine desulphurase;

a homocysteine assay which comprises contacting a biological fluid sample with a liquid reagent containing a homocysteine converting enzyme, wherein said reagent is produced by adding an aqueous liquid to a lyophilisate containing said enzyme and a cryo/lyoprotectant, characterised in that said lyophilisate is substantially free of thiol-containing cryo/lyoprotectants;

a homocysteine assay which comprises contacting a biological fluid sample with a liquid reagent containing homocysteine desulphurase, wherein said liquid reagent is an aqueous liquid containing homocysteine desulphurase, a thiol-reducing reagent, and a proteinaceous or non-proteinaceous stabilizer;

a homocysteine assay which comprises contacting a biological fluid sample with a homocysteine converting enzyme, characterised in that before contact with said enzyme said sample is treated with an agent which serves to deactivate pyruvates;

a homocysteine assay which comprises contacting a biological fluid sample with an immobilized homocysteine converting enzyme, wherein said biological fluid sample contacts the immobilized enzyme under such time and conditions to allow the homocysteine in the sample to bind to said enzyme, characterised in that the biological fluid sample is then removed from the assay; and

a homocysteine assay which comprises contacting a biological fluid sample with a homocysteine converting enzyme, characterized in that before contact with said enzyme said sample is filtered through an exclusion filter and centrifuged in order to remove pyruvates.

9. An assay which comprises at least four assays selected from the group consisting of:

an assay for homocysteine which comprises contacting a biological fluid sample with a reducing agent and subsequently with homocysteine desulphurase, characterised in

that said sample is contacted with an agent which binds, oxidizes or depotentiates said reducing agent after being contacted with said homocysteine desulphurase;

a homocysteine assay which comprises contacting a biological fluid sample with a liquid reagent containing a homocysteine converting enzyme, wherein said reagent is produced by adding an aqueous liquid to a lyophilisate containing said enzyme and a cryo/lyoprotectant, characterised in that said lyophilisate is substantially free of thiol-containing cryo/lyoprotectants;

a homocysteine assay which comprises contacting a biological fluid sample with a liquid reagent containing homocysteine desulphurase, wherein said liquid reagent is an aqueous liquid containing homocysteine desulphurase, a thiol-reducing reagent, and a proteinaceous or non-proteinaceous stabilizer;

a homocysteine assay which comprises contacting a biological fluid sample with a homocysteine converting enzyme, characterised in that before contact with said enzyme said sample is treated with an agent which serves to deactivate pyruvates;

a homocysteine assay which comprises contacting a biological fluid sample with an immobilized homocysteine converting enzyme, wherein said biological fluid sample contacts the immobilized enzyme under such time and conditions to allow the homocysteine in the sample to bind to said enzyme, characterised in that the biological fluid sample is then removed from the assay; and

a homocysteine assay which comprises contacting a biological fluid sample with a homocysteine converting enzyme, characterized in that before contact with said enzyme said sample is filtered through an exclusion filter and centrifuged in order to remove pyruvates.

10. An assay as claimed in claim 8 wherein said assay comprises:

an assay for homocysteine which comprises contacting a biological fluid sample with a reducing agent and subsequently with homocysteine desulphurase, characterised in that said sample is contacted with an agent which binds, oxidizes or depotentiates said reducing agent after being contacted with said homocysteine desulphurase;

a homocysteine assay which comprises contacting a biological fluid sample with a liquid reagent containing a homocysteine converting enzyme, wherein said reagent is

produced by adding an aqueous liquid to a lyophilisate containing said enzyme and a cryo/lyoprotectant, characterised in that said lyophilisate is substantially free of thiol-containing cryo/lyoprotectants; and

a homocysteine assay which comprises contacting a biological fluid sample with a homocysteine converting enzyme, characterised in that before contact with said enzyme said sample is treated with an agent which serves to deactivate pyruvates.

11. An assay as claimed in claim 8 wherein said assay comprises:

an assay for homocysteine which comprises contacting a biological fluid sample with a reducing agent and subsequently with homocysteine desulphurase, characterised in that said sample is contacted with an agent which binds, oxidizes or depotentiates said reducing agent after being contacted with said homocysteine desulphurase;

a homocysteine assay which comprises contacting a biological fluid sample with a liquid reagent containing homocysteine desulphurase, wherein said liquid reagent is an aqueous liquid containing homocysteine desulphurase, a thiol-reducing reagent, and a proteinaceous or non-proteinaceous stabilizer; and

a homocysteine assay which comprises contacting a biological fluid sample with a homocysteine converting enzyme, characterised in that before contact with said enzyme said sample is treated with an agent which serves to deactivate pyruvates.

12. An assay as claimed in claim 4 wherein the agent which serves to deactivate pyruvates is hydrogen peroxide.

13. An assay as claimed in claim 12 wherein the hydrogen peroxide is neutralized with catalase prior to contacting the sample with said homocysteine converting enzyme.

14. An assay as claimed in claim 4 wherein after the sample is treated with the agent, the sample is heated at 40-60°C for 15 to 60 minutes prior to contacting with said homocysteine converting enzyme.

15. An assay as claimed in claim 4 wherein the agent which serves to deactivate pyruvates is selected from the group consisting of pyruvate carboxylase, pyruvate oxidase, and lactate dehydrogenase.

16. (Cancelled)

17. (Cancelled)

18. An assay as claimed in claim 6 wherein the sample is filtered with a 30 kD exclusion filter.

19. An assay as claimed in claim 2 wherein said homocysteine converting enzyme is HDS and wherein a NAD^+/NADH cycling reaction is used to generate a coloured compound the concentration of which may be correlated to the concentration of homocysteine in the initial biological fluid sample.

20. A kit for a homocysteine assay, said kit comprising:
homocysteine desulphurase, preferably (i) in lyophilized form, the lyophilisate being substantially free of thio-containing cryo/lyoprotectants or (ii) in aqueous liquid form further containing a dithiol reducing agent;
a homocysteine or homocystine standard, preferably a plurality of standards containing HCy or homocytine at a plurality of known concentrations;
a reducing agent ;
an agent which binds, oxidizes or depotentiates the reducing agent;
optionally one or more further reagents capable of converting the homocysteine conversion product of homocysteine desulphurase into a detectable analyte;
preferably a pyruvate deactivating agent ; and
optionally a filter capable of removing red blood cells from blood.

21. An assay as claimed in claim 4 wherein said homocysteine converting enzyme is HDS and wherein a NAD^+/NADH cycling reaction is used to generate a

coloured compound the concentration of which may be correlated to the concentration of homocysteine in the initial biological fluid sample.

22. An assay as claimed in claim 5 wherein said homocysteine converting enzyme is HDS and wherein a NAD^+/NADH cycling reaction is used to generate a coloured compound the concentration of which may be correlated to the concentration of homocysteine in the initial biological fluid sample.

23. An assay as claimed in claim 6 wherein said homocysteine converting enzyme is HDS and wherein a NAD^+/NADH cycling reaction is used to generate a coloured compound the concentration of which may be correlated to the concentration of homocysteine in the initial biological fluid sample.

APPENDIX B

MARKED UP COPY OF CLAIMS

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a homocysteine assay which comprises contacting a biological fluid sample with a homocysteine converting enzyme, characterized in that before contact with said enzyme said sample is filtered through an exclusion filter and centrifuged in order to remove pyruvates.

8. (Amended) An assay [as claimed in] which comprises at least three [of claims 1 to 6] assays selected from the group consisting of:

an assay for homocysteine which comprises contacting a biological fluid sample with a reducing agent and subsequently with homocysteine desulphurase, characterised in that said sample is contacted with an agent which binds, oxidizes or depotentiates said reducing agent after being contacted with said homocysteine desulphurase;

a homocysteine assay which comprises contacting a biological fluid sample with a liquid reagent containing a homocysteine converting enzyme, wherein said reagent is produced by adding an aqueous liquid to a lyophilisate containing said enzyme and a cryo/lyoprotectant, characterised in that said lyophilisate is substantially free of thiol-containing cryo/lyoprotectants;

a homocysteine assay which comprises contacting a biological fluid sample with a liquid reagent containing homocysteine desulphurase, wherein said liquid reagent is an aqueous liquid containing homocysteine desulphurase, a thiol-reducing reagent, and a proteinaceous or non-proteinaceous stabilizer;

a homocysteine assay which comprises contacting a biological fluid sample with a homocysteine converting enzyme, characterised in that before contact with said enzyme said sample is treated with an agent which serves to deactivate pyruvates;

a homocysteine assay which comprises contacting a biological fluid sample with an immobilized homocysteine converting enzyme, wherein said biological fluid sample contacts the immobilized enzyme under such time and conditions to allow the homocysteine in the sample to bind to said enzyme, characterised in that the biological fluid sample is then removed from the assay; and

a homocysteine assay which comprises contacting a biological fluid sample with a homocysteine converting enzyme, characterized in that before contact with said enzyme said sample is filtered through an exclusion filter and centrifuged in order to remove pyruvates.

9. (Amended) An assay [as claimed in] which comprises at least four [of claims 1 to 6] assays selected from the group consisting of:

an assay for homocysteine which comprises contacting a biological fluid sample with a reducing agent and subsequently with homocysteine desulphurase, characterised in that said sample is contacted with an agent which binds, oxidizes or depotentiates said reducing agent after being contacted with said homocysteine desulphurase;

a homocysteine assay which comprises contacting a biological fluid sample with a liquid reagent containing a homocysteine converting enzyme, wherein said reagent is produced by adding an aqueous liquid to a lyophilisate containing said enzyme and a cryo/lyoprotectant, characterised in that said lyophilisate is substantially free of thiol-containing cryo/lyoprotectants;

a homocysteine assay which comprises contacting a biological fluid sample with a liquid reagent containing homocysteine desulphurase, wherein said liquid reagent is an aqueous liquid containing homocysteine desulphurase, a thiol-reducing reagent, and a proteinaceous or non-proteinaceous stabilizer;

a homocysteine assay which comprises contacting a biological fluid sample with a homocysteine converting enzyme, characterised in that before contact with said enzyme said sample is treated with an agent which serves to deactivate pyruvates;

a homocysteine assay which comprises contacting a biological fluid sample with an immobilized homocysteine converting enzyme, wherein said biological fluid sample contacts the immobilized enzyme under such time and conditions to allow the homocysteine in the sample to bind to said enzyme, characterised in that the biological fluid sample is then removed from the assay; and

a homocysteine assay which comprises contacting a biological fluid sample with a homocysteine converting enzyme, characterized in that before contact with said enzyme said sample is filtered through an exclusion filter and centrifuged in order to remove pyruvates.

10. (Amended) An assay as claimed in [claims 1, 2, and 4] claim 8 wherein said assay comprises

an assay for homocysteine which comprises contacting a biological fluid sample with a reducing agent and subsequently with homocysteine desulphurase, characterised in

that said sample is contacted with an agent which binds, oxidizes or depotentiates said reducing agent after being contacted with said homocysteine desulphurase;

a homocysteine assay which comprises contacting a biological fluid sample with a liquid reagent containing a homocysteine converting enzyme, wherein said reagent is produced by adding an aqueous liquid to a lyophilisate containing said enzyme and a cryo/lyoprotectant, characterised in that said lyophilisate is substantially free of thiol-containing cryo/lyoprotectants; and

a homocysteine assay which comprises contacting a biological fluid sample with a homocysteine converting enzyme, characterised in that before contact with said enzyme said sample is treated with an agent which serves to deactivate pyruvates.

11. (Amended) An assay as claimed in [claims 1, 3, and 4] claim 8 wherein said assay comprises

an assay for homocysteine which comprises contacting a biological fluid sample with a reducing agent and subsequently with homocysteine desulphurase, characterised in that said sample is contacted with an agent which binds, oxidizes or depotentiates said reducing agent after being contacted with said homocysteine desulphurase;

a homocysteine assay which comprises contacting a biological fluid sample with a liquid reagent containing homocysteine desulphurase, wherein said liquid reagent is an aqueous liquid containing homocysteine desulphurase, a thiol-reducing reagent, and a proteinaceous or non-proteinaceous stabilizer; and

a homocysteine assay which comprises contacting a biological fluid sample with a homocysteine converting enzyme, characterised in that before contact with said enzyme said sample is treated with an agent which serves to deactivate pyruvates.

12. An assay as claimed in claim 4 wherein the agent which serves to deactivate pyruvates is hydrogen peroxide.

13. (Amended) An assay as claimed in claim 12 wherein the hydrogen peroxide is [neutralised] neutralized with catalase prior to contacting the sample with said homocysteine converting enzyme [using catalase].

14. (Amended) An assay as claimed in [any one of claims] claim 4[, 12 or 13] wherein after the sample is treated with the [said] agent, the sample is heated at 40-60°C for 15 to 60 minutes prior to contacting with said homocysteine converting enzyme.

15. (Amended) An assay as claimed in claim 4 wherein the agent which serves to deactivate pyruvates is selected from the group consisting of pyruvate carboxylase, pyruvate oxidase, and lactate dehydrogenase.

16. (Cancelled)

17. (Cancelled)

18. An assay as claimed in claim 6 wherein the sample is filtered with a 30 kD exclusion filter.

19. (Amended) An assay as claimed in [any one of claims 1 to 18] claim 2 wherein said homocysteine converting enzyme is HDS and wherein a NAD^+/NADH cycling reaction is used to generate a coloured compound the concentration of which may be correlated to the concentration of homocysteine in the initial biological fluid sample.

20. (Amended) A kit for a homocysteine assay, said kit comprising:
homocysteine desulphurase, preferably (i) in lyophilized form, the lyophilisate being substantially free of thio-containing cryo/lyoprotectants or (ii) in aqueous liquid form further containing a dithiol reducing agent [(e.g. DTT, DTE or TCEP) and a proteinaceous or non-proteinaceous stabilizer];

a homocysteine or homocystine [homocyst (e) ine] standard, preferably a plurality of standards containing HCy or homocytine at a plurality of known concentrations;

a reducing agent [, e.g. dithiothreitol, dithioerythiol, TCEP or methyl iodide];

an agent which binds, oxidizes or depotentiates the reducing agent [, e.g. an organic disulphide or a dithiol binding agent, preferably a maleimide];

optionally one or more further reagents capable of converting the homocysteine conversion product of homocysteine desulphurase into a detectable analyte;
preferably a pyruvate deactivating agent [, e.g. hydrazine, acetoacetate decarboxylase, pyruvate carboxylase, hydrogen peroxide or pyruvate dehydrogenase];
optionally a filter for removing pyruvate [, i.e. a molecular sieve]; and
optionally a filter capable of removing red blood cells from blood.

21. (New) An assay as claimed in claim 4 wherein said homocysteine converting enzyme is HDS and wherein a NAD^+ / NADH cycling reaction is used to generate a coloured compound the concentration of which may be correlated to the concentration of homocysteine in the initial biological fluid sample.

22. (New) An assay as claimed in claim 5 wherein said homocysteine converting enzyme is HDS and wherein a NAD^+ / NADH cycling reaction is used to generate a coloured compound the concentration of which may be correlated to the concentration of homocysteine in the initial biological fluid sample.

23. (New) An assay as claimed in claim 6 wherein said homocysteine converting enzyme is HDS and wherein a NAD^+ / NADH cycling reaction is used to generate a coloured compound the concentration of which may be correlated to the concentration of homocysteine in the initial biological fluid sample.